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Effects of Rumen-Protected Amino Acids on Production in Holstein Cows

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EFFECTS OF RUMEN-PROTECTED AMINO ACIDS ON PRODUCTION IN HOLSTEIN COWS

by

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Paul Dawson

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ABSTRACT

Effects of Rumen-Protected Amino Acids
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by

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Six nonlactating Holstein cows fitted with rumen and duodenal cannula were used to determine the efficacy of pH sensitive fatty acid polymer encapsulation as a means protecting amino acids from rumen fermentation and as a post-ruminal amino acid delivery system. The cows were arranged in a 2 X 2 factorial in a Latin Square design. Treatments were 1) the basal ration, and 2) basal ration plus rumen-protected lysine, methionine, and threonine at 10 g each per day.

Rumen parameters measured were pH, ammonia, volatile fatty acids, protozoa, liquid, and dry matter rate of passage, total viable bacteria, and viable cellulolytic bacteria. Duodenal parameters measured were crude protein, ammonia, and amino acid concentrations. Total tract apparent digestibility of nutrients was measured.

In addition, rumen degradation of the three amino acid products was measured by loss from nylon bags, in the rumen.

Loss of product from nylon bags suggested the lysine and threonine products had no significant rumen protection, but that the methionine product had > 50% protection at 12 h in the rumen. None of the rumen parameters measured differed ($P > .05$) due to treatment. Duodenal crude protein and ammonia concentrations did not differ due to treatment. Duodenal amino acid concentrations were numerically higher for the amino acid supplemented treatment, but the differences were nonsignificant and thought to be confounded by failure of the lysine and threonine products. Total tract apparent nutrient digestibility was not affected by treatment.

A second experiment was conducted using 40 post-parturient Holstein cows, and different rumen-protected amino acid products from the first trial. Cows were nesteMwby treatment (control vs rumen-protected methionine 46 g and lysine 22 g) and parity (primiparous vs multiparous).

Dry matter intake and milk production were monitored daily, body weight weekly, and milk composition bi-weekly. Total tract apparent nutrient digestibility was measured during the last week of the 10-week trial.

There was no significant ($P < .05$) effect of supplemental rumen-protected lysine and methionine among primiparous animals. Multiparous animals receiving supplemental amino acids had lower dry matter intakes and yield of milk components than control animals.

(85 pages)

CHAPTER I

INTRODUCTION

In most domestic monogastric animals the metabolic amino acid (AA) requirements have been established. It is common practice to add specific AA such as lysine and methionine to swine and poultry rations. By making more efficient use of feed proteins, producers are able to reduce the total amount of the expensive protein fraction in the diet. In addition, there is a reduced chance of detrimental effects associated with feeding large amounts of protein (39).

In order to maximize milk protein yield, the mammary gland has to have available precursors in sufficient quantities (15, 54). Supply of precursors and hormones interact to determine actual milk production. This paper is primarily concerned with the supply of milk protein precursors in ruminants.

It is recognized that feeding supplemental non-fermentable protein (UIP) is sometimes more efficient at increasing the quantity of AA available to ruminants than feeding fermentable protein (DIP). This is particularly so when feeding moderate to high levels of fermentable feeds (51). The biological value of protein, both rumen undegraded intake protein (UIP) and rumen degraded intake protein (DIP), is determined by availability of the limiting AA. Thus, the ability to supply small quantities

of specific AA to the small intestine should allow improved protein efficiency. By encapsulating AA in a polymer, the structure of which is pH dependent, it is possible to protect the AA from microbial degradation in the rumen and allow polymer breakdown in the abomasum. This is possible because the polymer forms a stable capsule at the pH of the rumen (pH 5-7), but is destabalized at the pH of the abomasum (pH 1.5-2.5).

The objectives of this study were to determine the effects of specific polymer-encapsulated lysine and methionine on 1) nutrient digestion and 2) milk production.

CHAPTER II

LITERATURE REVIEW

Nitrogen in the Rumen

The Crude Protein System

The pregastric fermentation of feeds in the ruminant has, until recently, resulted in less emphasis on specific AA. The 1978 National Research Council, Subcommittee on Dairy Cattle Nutrition, expressed protein requirements in terms of CP (crude protein = N X 6.25)(50). This system reflected the belief that microbial fermentation largely degrades feed protein to provide nutrients for microbial growth. The microbial protein resulting from fermentation is the major source of AA in the small intestine. Thus, why look at the AA content of a feed when microbial fermentation changes AA profiles before they are rendered available to the animal? Microbial degradation of fermentable protein is not linked to microbial requirements for ammonia nitrogen (NH3-N). Any excess NH3-N formed leaves the rumen by absorption into the blood or passage to the abomasum and (with the exception of some recycling to the rumen via the saliva) and is largely excreted by the cow. As a result, feeding increased amounts of fermentable protein only increases the AA supply to the cow to the point where rumen microbes have

sufficient NH3-N for growth. This does not mean it does not matter how much fermentable protein is fed. Rumen microbial activity is stimulated by increases in fermentable CP when CP is deficient. Clark and Davis (16) refer to data that suggest a 1% increase in DM digestibility with each 1% increase in CP content of the diet from 12 to 18%.

In order to maximize rumen microbial productivity, it is necessary to provide the right environment. There are a large number of studies evaluating conditions in the rumen and how these relate to microbial activity. For example, NH3-N and energy, and their availability profiles, pH, lipids, and the presence of adequate sulfur and branched chain AA have all been shown to be important in the fermentative process (1, 6, 14, 16, 25, 30, 35, 40, 51, 52, 77, 83). Of particular interest in protein metabolism is the role of rumen NH3-N. Assuming adequate availability of other substrates, an NH3-N concentration of 3 to 5 mg/dl of rumen fluid has been shown to be adequate for maximal microbial activity (18, 40, 43, 52, 64). Actual rumen NH3-N concentration ranges from 1 to 76 mg/dl (52), suggesting that both deficiencies and excesses do occur.

Microbial fermentation undoubtedly complicates dietary formulation on a component basis. We must,

therefore, always remember the unique benefits of microbial digestion. The principal advantage of microbial digestion is the digestion of structural carbohydrates. Cellulose and starch are very similar in composition, so it is not surprising that both have a similar gross energy content. Big differences in net energy lie in the fact mammals do not produce cellulase and are consequently unable to utilize cellulose without the aid of microbial digestion, such as occurs in the rumen (74).

The UIP/DIP System

When requirements for production exceed the supply from rumen fermentation, deficiencies have to Mw met from body stores or from material escaping rumen fermentation (52). Coppock et al. (20) calculated that labile protein reserves could support a maximum of 126 kg milk, while fat reserves could provide energy for 1000 kg milk. Milk protein precursors have to come largely from the gastrointestinal (GI) tract. As our understanding of the reticulo-rumen improves, ways are being found to augment its function.

Rumen-escape, rumen-bypass, rumen-unavailable, and rumen-protected are terms used to describe material that avoids microbial digestion. The term used by the NRC is undegraded intake protein (UIP). The term rumen-protected protein describes material that is shielded from microbial

degradation, either chemically or physically (2, 26, 33, 44, 68, 84). Ways in which material can avoid microbial degradation have been reviewed by numerous authors (9, 40, 57). They include naturally resistant proteins, such as those found in corn, fish meal, meat meal, and brewer's byproducts. Alternatively, proteins which would normally be rapidly degraded in the rumen can be rendered resistant; heat, tannin, acetic acid, and formaldehyde have all been used successfully (9, 16, 22, 40, 66, 76).

During the 1980's, NRC concluded that high-producing cows require integrated rumen and nonrumen digestion in order to maximize AA supply to the small intestine (51). This led to the development of the UIP/DIP protein system (51). This system differs from the CP system in that feed proteins are classified as either rumen degraded intake protein (DIP), or rumen undegraded intake protein (UIP). Formulation of rations using UIP and DIP requirements has allowed for a reduction in the recommended protein concentration in the ration. For example, a 680 kg cow that is producing 59 kg of 3.5% fat milk and consuming 4.5% of its body weight (BW) will require 22.0% CP using CP requirements only, compared to 16.7% CP using UIP and DIP requirements (51).

Material escaping ruminal degradation is only useful if it is subsequently absorbed. There is some evidence suggesting a negative correlation between resistance to ruminal degradation and availability in the small intestine (9, 16, 22). Feed processors have to be careful that processing does not render feed indigestible (2, 66, 76).

As mentioned earlier, a rumen NH3-N concentration of 3 to 5 mg/dl is thought to be adequate for optimal rumen activity. Diets consisting of unprocessed feeds (whole plant proteins) with 13% CP (approximately 70% DIP) generally provide 3 to 5 mg/dl rumen NH3-N (40, 43, 52, 63). Thus, cows whose requirements are greater than 13% CP should be receiving increasing proportions of their protein from UIP.

Microbial Crude Protein Production

The dietary content of DIP, minerals, branched chain fatty acid, etc. have to be adequate, but intake of fermentable carbohydrates is the most important determinant of microbial activity (1, 51). The United Kingdom (UK) Agricultural Research Council (1) suggests values of 1.34 g microbial nitrogen produced per MJME, or 32 g microbial CP per kg organic matter digested in the rumen. Thus, assuming other nutrients are not deficient, it is possible to estimate the microbial AA supply to the

small intestine using rumen digestible organic matter intake.

It is important to stress the necessity of maintaining "optimal rumen activity." A number of studies have failed to show the expected benefit from increasing the proportion of UIP because of a concomitant reduction in microbial activity and microbial CP (18, 27, 36, 38, 48, 66, 67, 85). Substituting nonprotein nitrogen (NPN) and UIP for normally degradable natural protein has often failed to result in increased milk production (67). There are a number of possible reasons for this: 1) deficiency of microbial growth factors normally found in natural protein (sulfur for synthesis of methionine and cysteine; and branched chain AA for the synthesis of essential fatty acids), and 2) mismatching of ammonia and energy availability profiles (NPN tends to be degraded within 1 h, resulting in periodic ammonia deficiencies) (35, 77).

Amino Acids in the Small Intestine

Microbial Amino Acids

The AA content and digestibility of microbial protein has been studied by numerous authors (70). Some authors conclude there are no significant differences in the AA content of mixed microbial proteins (70). Others claim that significant differences in AA content of rumen

microbes do occur (19). It is important to consider a number of items when assessing conflicting data in this area, including: 1) different techniques of sample collection and analyses, and 2) improvements in analytical technique means data analyzed prior to 1970 should be treated as unreliable (23). It seems likely there will be some differences in mixed rumen microbial AA content associated with dietary feeding regimes. However, for the present, an average microbial AA composition that is derived from a range of recent experiments will probably give the best estimate of microbial AA composition. Microbial protein appears to be relatively rich in essential AA, when compared to plant proteins.

UIP AA Supply

It is becoming increasingly clear that AA profile of feed available in the small intestine needs to be considered. Rumen-escape protein is only useful to the extent that it supplies limiting AA. For example, when lysine is the limiting AA, soybean meal (SBM) UIP would have ten times the metabolic value of corn gluten feed UIP (52). This illustrates one of the problems associated with the DIP/UIP system. The most controlled means of supplying a specific AA to the small intestine is as rumen-protected AA (RPAA). Amino acids are protected by encapsulation in a pH sensitive fatty acid polymer (57).

The fatty acid polymer shields the AA from degradation in the mildly acidic rumen, while a structural change releases it to the more acidic abomasum for absorption in the small intestine.

The Amino Acid System

A ruminant feeding system using AA requirements will have to differentiate between feed nitrogen available in the rumen and AA available in the small intestine. Several such systems have been proposed (52, 53, 63, 67). The current NRC and ARC (1, 51) feeding systems differentiate between rumen degradable and rumen escape proteins. However, because of insufficient data, neither system extends to AA. The metabolic value of feed protein is complex, but can be divided into three fractions (38, 41, 46): 1) a rapidly rumen degraded fraction (0% metabolically available as feed protein, though the nitrogen may be incorporated into microbial CP), 2) the undigestible N (0% metabolically available, part of the fecal-N)(52), and 3) the insoluble available N. Proteins that are insoluble, but available, undergo digestion either in the rumen or postruminally. The metabolic value of this fraction is related to the partitioning of digestion between the rumen (nitrogen to NPN or microbial CP), the small intestine (AA absorbed directly), and the hind gut (nitrogen to microbial protein, part of the

fecal-N) (41). There are means of estimating the relative quantities of each fraction (40, 46, 52, 56).

In theory, there is sufficient understanding to identify and correct probable AA deficiencies. The simplest and most efficient means to modify the amount of a specific AA is by feeding a rumen-protected amino acid (RPAA). As an understanding of ruminant nutrition improves, the formulation of rations on an AA basis will be developed.

Amino Acid Requirements

In order to match nutrient supply with demand it is necessary to know the animal's requirements. The same amino acids are regarded as essential for ruminant and monogastric animals (7, 29); however, the amount of each AA required for maintenance, growth, or production is unknown. There are several ways researchers have investigated the AA status of lactating dairy cows. A simple method is to add AA by intravenous or postruminal infusion. This has resulted in increases in milk protein production of 10 to 15% (15). The major problem with this technique is not knowing the AA supplied by the rest of the gastro-intestinal tract (GIT). Thus, researchers are unable to say more than amino acids are limiting under the conditions of the experiment. An alternative is to

monitor changes in plasma AA profiles with changes in AA supply (8). Thus, if the supply of an AA increases, but there is no accompanying increase in its plasma concentration, then it is suggested that the AA was limiting and is being taken up by increased protein synthesis. For the same reason, an increased supply of a limiting AA should result in a decreased concentration of other AA. Again without estimates for AA contributed by the GIT, results are of limited application. Other methods include: 1) differences in the AA content of milk and digesta, or 2) arterial and venous blood across the mammary gland (4, 5, 11, 12, 15, 43, 53, 57, 60, 61, 67, 83). No one technique has received universal acceptance because each has inherent problems. Intragastric nutrition has overcome some of the problems associated with rumen fermentation. In this procedure, rumen fermentation is replaced by continuous infusion of a cocktail of fermentation products (55). By manipulating the infusate, researchers have been able to estimate the digestibility and requirement for microbial protein at nitrogenous equilibrium (72), and the optimum profile of the five limiting amino acids in microbial protein for tissue maintenance (71). This allows maintenance AA requirements to be estimated.

Milk protein production is directly affected by the

AA concentration in the blood, mammary gland blood flow, and carrier systems (mainly RNA) to transport AA across cell membranes. Details are discussed in extensive reviews by Clark et al. (17), Mephan (47), and Waghorn and Baldwin (78).

There are a number of candidates for the limiting or co-limiting AA e.g., lysine, methionine, tryptophan, threonine, phenylalanine, histidine, and leucine (15, 23, 40, 43, 60, 66, 67). The most consistant positive response has been with lysine, used in early lactation, when corn provides a significant portion (> 20-25%) of the protein in the diet (27, 67, 77). Under similar conditions, when soybean meal (SBM) is used for UIP, methionine appears to be first limiting (11, 68, 75). Combinations of these common feedstuffs may result in either or both of these amino acids being limiting (60, 61).

A point worth emphasizing is that maximal protein stress, like other nutrients, is within the first 10 wk of lactation. At this time the cow's intake lags behind production requirements and the cow is consequently utilizing body stores (40, 64, 77). Furthermore, protein stress, like other nutrients, is greatest in high-producing animals. Production and intake are highly variable in early lactation. High innate variability,

together with the lack of a precise baseline value for milk production, means relatively large numbers of cows are needed per treatment in this type of study (65).

CHAPTER III

MATERIAL AND METHODS

Experiment 1. Effects of RPAA on Digestion

Experimental Design

Six mature, nonlactating, nonpregnant Holstein cows equipped with rumen and duodenal cannulae were bedded on wood shavings, individually fed, housed, and allowed free access to water and trace mineralized salt blocks. Cows were fed twice daily 10 kg of a semipurified diet (Table 1). In order to magnify the effects of the relatively small quantities of supplemental treatment RPAA, the basal ration contained minimal true protein. Cows were randomly assigned to one of the two treatments. The treatments were either the basal ration or the basal ration plus 10 g of each RPAA. The six cows were arranged in a 2 X 2 factorial, with a Latin square design, with three replications. The three rumen-protected AA products, BYlys^{R}, BYmet^{R}, and BYthr^{R} contained 35% L-lysine, DL-methionine, or L-threonine, respectively (manufacturer's guaranteed analysis)(Animal Technology, Inc., 41593 Winchester Road, Suite F, Temecula, CA 92390). The RPAA products, 5 g each, were applied as a top dressing at each feeding. Experimental periods consisted of 14 d dietary

TABLE 1. Composition of the basal ration for experiment 1.

Item	% DM
Wheat straw	25.0
Corn starch	32.7
Dextrose	32.2
Urea (45% N)	3.67
Molasses	2.25
Minerals and vitamins[1]	3.75
Sulfur	0.08
Choline chloride (50%)	0.37
Component	
Crude protein	11.49
ADF	14.80
NDF	25.80
Ash	3.52

[1] Consisted of .5% Mn, .5% Zn, .5% Fe, .45% Ca, .05% Cu, .015% I, .01% Co, 36,400 IU/kg of vitamin A, 180 IU/kg of vitamin E.

adaptation, followed by 3 d sample collection. After the first experimental period, treatments were switched and the procedure was repeated.

Insacco DM Disappearance

Two of the cows on the control ration were used to measure insacco DM disappearance, following sample collection for digestive parameters. Insacco rumen DM disappearance rates were determined for the RPAA products according to the following procedures: Approximately 1 g of RPAA product was quantitatively weighed into each of 16 polyester bags [10 X 10 cm, pore size 60 μm ("Elite White" Lucern fabrics N.Y., NY)]. Bags were sewn closed and 14 of the bags were placed in a large weighted bag in the rumen. The two remaining bags were used to determine loss during the washing procedure. Two bags each were removed at 1, 2, 4, 8, 12, 24 and 48 h residence time in the rumen and immediately frozen (-10 °C) until all bags had been collected. All 16 bags were machine washed as directed by Cherney et al. (13). Washed bags were frozen (-10 °C) until lyophilized and reweighed.

Sample Collection and Analysis

The basal ration was subsampled weekly. Feed samples were dried (72 h at 60 °C), ground through a 1-mm screen using a Wily mill (Thomas Wiley Laboratories, Suedesboro,

NJ), composited by weight within experimental period, and analyzed for ADF (3), NDF (59), CP (37), DM and ash (3). For the 3 d collection period rumen, duodenal and fecal samples were collected. Rumen and duodenal digesta were collected at 0, 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h postfeeding. After the time 0 digesta collection and prior to feeding, cows were dosed, via the rumen cannula, with 100 g Cr-mordanted straw and 54 g LiCo-EDTA. Rumen contents were mixed by hand prior to each sampling. Immediately upon collection both rumen and duodenal digesta were placed in an insulated container with freeze packs. The pH (Fisher Accumet^{R} Model 425 digtal pH/Ion meter, 711 Forbes Avenue, Pittsburg, PA 15219) of the rumen digesta was measured in the first seven samples (0-12 h). A subsample of rumen digesta was strained through 4 layers of cheese-cloth, preserved by the addition of 10% 6 N HCl and stored frozen (-10 °C). The preserved rumen fluid was centrifuged at 20,000 X g for 10 min and the supernatant collected for analysis of volatile fatty acids (VFA) by gas chromatography (HP 5890 Hewlett-Packard Company, Analytical Group #10224, P.O. Box 9000, San Fernando, CA 91341-9981), cobalt by atomic absorption spectrophotometry (Buck Scientific Incorporated 58 Fort Point Street, E. Norwalk, CT 06855) and ammonia nitrogen (37). The remaining rumen digesta was frozen (-10 °C)

until lyophilized. Lyophilized rumen samples were ground through a cyclone grinder (Cyclotec 1093 sample mill, Tecator AB, P.O. Box 70. S-263 21 Hoganas, Sweden) and the chromium concentration was measured by atomic absorption spectrophotometry (82). Total number of protozoa (80), viable cellulolytic bacteria, and viable total bacteria in rumen fluid were measured in the 4 h postfeeding sample, using the differential media and methods of Leedle and Hespell (45).

A subsample of the first seven samples (0-12 h) of duodenal digesta was preserved by the addition of 10% 6 N HCl, centrifuged at 20,000 X g, and the supernatant frozen (-10 °C) until analyzed for ammonia nitrogen (37). The remaining duodenal digesta was frozen (-10 °C) until lyophilized. Lyophilized duodenal samples were ground through a 1-mm screen using a Wiley mill and analyzed for CP (37). Lyophilized duodenal samples were composited by weight within cow within period and analyzed for AA by HPLC, using the following proceedures: A sample of known (at least approximately) protein concentration is weighed into a glass ampule; 6 N HCL are added to odtain a concentration of about 5 mg protein per ml 6N HCL (49, 65). The ampule is placed in an ultrasonic cleaner and the oxygen removed by alternating between vacuum and nitrogen gas for about 4 min (42, 49, 65). The ampule is

heat sealed and placed in a heating block at 110 °C for 20 h (28, 49, 58, 65). After hydrolysis, ampules are removed from the heating block. The hydrolysate is filtered through a 0.2-μm filter to remove particulates. An aliquot (generally 10-20 μl) of the hydrolyzate is dried under nitrogen gas. The dried sample is dissolved in 250 μl sample buffer prior to filtering through a 0.2-μm filter. The sample is loaded into a sample cassette and placed in a Beckman 6300 High Performance Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, CA). When looking at the results of the AA analysis, not all the amino acids are present. Glutamine and asparagine are converted to glutamic and aspartic acids, respectively. Tryptophan is completely destroyed by acid hydrolysis. Cysteine is easily oxidized and lost during hydrolysis. Methionine may also be oxidized. Lysine may be lost by Maillard browning reactions. Serine, threonine, and tyrosine are partially destroyed. Valine, leucine, and isoleucine are not completely hydrolyzed. The rates at which amino acids are destroyed depend on acid concentration, time, and temperature of hydrolysis; specific proteins; and the presence of carbohydrates, aldehydes, and metal impurities (21, 58, 79).

Fecal samples were collected morning and afternoon for the 3 d collection period, and frozen (-10 °C) until

dried (72 h at 60 °C). Dry fecal samples were ground through a 1-mm screen using a Wiley mill and composited within cow, within period, prior to analysis for ADF (3), NDF (59), CP (37), DM, and ash (3).

Statistical Analysis

Data were analyzed as a 2 X 2 factorial arranged in a Latin square, replicated three times, using the general linear models of SAS (62). The model used was

$$Y = \mu + T + C + P + Tm + T*Tm$$

where Y is the dependent variable, μ the mean, T the treatment, C the cow, P the period, Tm the time of repeated measures, and T*Tm the treatment by time interaction. Significance was declared at $P < .05$.

Experiment 2. Effects of RPAA on Milk Production

Experimental Design

Forty postparturient Holstein cows, housed at the Utah State University Caine Dairy Center, were blocked by parity (first or > first) and assigned to one of two treatment diets. The dietary treatments were (control) total mixed ration (TMR), or (supplemental RPAA) TMR + 92 g rumen-protected methionine ($BYMet^R$) + 43 g rumen-protected lysine ($BYLys^R$)(guaranteed analysis, minimum 50% amino acid). The TMR (Table 2) was formulated according

to NRC (51) recommendations for a 630 kg cow producing 37 kg 3.5% FCM, while losing 0.5 kg BW/d, with 50:50 primiparous:multiparous averaging 40 DIM (values predicted from previous trials). Corn supplied approximately 20% of the CP in the TMR, which was the lower level at which previous work suggested lysine became limiting. Treatment of all cows was the same except for the supplemental RPAA, which was applied as a top dressing and mixed with the morning feed. Cows were fed TMR twice daily to appetite plus 5 kg (as fed), with individual intakes monitored by use of Calan gates (American Calan, Inc., Northwood, NH).

Estimating AA Requirements

Estimate of Maintenance AA Requirements. A crude estimate of the AA requirement for maintenance of cows (Table 3) was obtained by multiplying the maintenance requirement for microbial CP at the duodenum of beef cows (5.01 g N/kg $BW^{0.75}$)(72) by the AA content of microbial CP (0.80)(70), by the digestibility of microbial AA in sheep (0.85)(72), by the proportion of each AA in microbial protein (0.0812 for lysine)(70), by the proportion of that AA required to provide the optimum AA profile for maintenance in sheep (0.946 for lysine)(71), and by $BW^{0.75}$. For example, the estimated maintenance requirement for lysine for animals weighing 580 kg (the estimated BW of

TABLE 2. Composition of the basal ration for experiment 2.

Item	% DM
Corn silage	12.00
Alfalfa hay (28% ADF)	27.00
Alfalfa haylage (32% ADF)	4.98
Beet pulp w/ molasses	11.04
Ground corn	8.18
Ground barley	8.18
Wheat bran	8.01
Cottonseed meal	7.61
Distillers corn grain	7.52
Molasses	1.50
Animal fat	1.98
Minerals and vitamins[1]	2.00
Nutrient composition	
Crude protein	17.1
UIP[2]	6.1
ADF	26.8
NDF	51.4
Ash	10.3

[1] .5% Mn, .5% Zn, .5% Fe, .45% Ca, .05% Cu, .015% I, .01% Co, 36,400 IU/kg of vitamin A, 180 IU/kg of vitamin E.

[2] Estimated using NRC (51) values.

animals to be used in this trial) is:

5.01 X 0.80 X 0.85 X 0.0812 X 0.946 X 126 = 33.24 g

Estimated AA Requirements for Milk Production. A simple estimate of AA requirements for milk production is the milk AA content. This does not account for transamination within the mammary gland, but it may be argued that such transaminations represent deviations from the optimal AA supply. The estimated AA requirements for 37 kg of milk containing 3.1% CP (production estimated from previous trials) are in Table 4.

AA Supply

Estimated Microbial AA Supply. Rumen microbial AA production was estimated by multiplying the microbial nitrogen production (1.25 g N/MJME) (excluding fat)(81) by the energy intake MJME (excluding fat)(286.9 MJME), by the AA content of microbial N, by 6.25 to convert nitrogen to CP, and by the individual AA content of mixed rumen microbial CP (70) (Table 5).

Estimated Feed AA Supply. By using individual feed UIP (51) and AA values (32), it is possible to estimate the undegraded feed AA supply to the duodenum (Table 6).

Rumen microbial amino acids have an availability of 0.85 (72). The availability of UIP can be estimated by subtracting the indigestible ADF-N fraction from the total UIP (34). For the ingredients used in this study,

TABLE 3. Estimated amino acid requirements for maintenance of a cow with a metabolic BW of 126 kg.

Amino Acid	Requirement g/hd/d
Cysteine	3.50
Valine	18.70
Phenylalanine	19.09
Arginine	18.17
Histidine	6.35
Isoleucine	18.83
Leucine	25.75
Lysine	33.24
Methionine	10.69
Threonine	18.00

TABLE 4. The amino acid requirement for 37 kg/d milk at 3.1% CP[1].

Amino Acid	Requirement g/hd/d
Cysteine	9.56
Valine	77.66
Phenylalanine	57.35
Arginine	40.62
Histidine	29.87
Isoleucine	69.30
Leucine	113.51
Lysine	94.39
Methionine	29.87
Threonine	53.77

[1] Amino acid content of milk protein from Kaufmann (43).

TABLE 5. The microbial amino acid supply to the duodenum when feeding 287 MJME.

Amino Acid	Supply g/hd/d
Cysteine	18.93
Valine	101.11
Phenylalanine	103.19
Arginine	93.34
Histidine	32.19
Isoleucine	103.00
Leucine	140.87
Lysine	153.74
Methionine	46.77
Threonine	98.46

TABLE 6. Estimated rumen undegraded feed amino acids in the duodenum.

Amino Acid	Supply g/hd/d
Cysteine	16.71
Valine	61.68
Phenylalanine	54.88
Arginine	75.27
Histidine	27.10
Isoleucine	46.59
Leucine	93.38
Lysine	49.62
Methionine	19.42
Threonine	48.10

duodenal availability estimates range from 0.6 for alfalfa hay to > 0.9 for CSM (41). The ARC (1) suggests a value of 0.7 for UIP duodenal availability, which is close to the average of the suggested digestibilities of feeds in this study (0.75). Using values of 0.7 and 0.85 for feed UIP AA and microbial AA availability, respectively, it is possible to estimate the total amino acids available for

production in the cow (Table 7). Also in Table 7 is the estimated total AA requirement of a 580 kg cow producing 37 kg of milk containing 3.1% milk protein, and a comparison between AA supply and requirements.

Data Collection and Analysis

Cows were weighed weekly throughout the trial. Individual cow milk samples were collected without preservative and composited from an a.m. and p.m. milking each week and again composited to provide a bi-weekly sample for analysis of total protein, casein protein, whey protein, and nonprotein nitrogen (3). Additionally, bi-weekly a.m.-p.m. composite milk samples were collected and preserved with potassium dichromate prior to analysis for fat, protein, lactose, and solids-non-fat (SNF) percent by infrared analysis (DHIA, Logan, UT) using a Multispec Infrared Analyzer (Wheldrake, Yorkshire, England). TMR and orts samples were collected weekly, dried at 60 °C for 72 h, and ground to pass through a 1-mm screen using a Wiley mill. TMR and ort samples were composited by weight on a monthly basis and the composite was analyzed for DM (105°C overnight), CP (34), ADF (3), NDF (59), and acid insoluble ash (AIA) (73).

TABLE 7. Available amino acid supply, requirements, and the difference (g/h/d) for a cow producing 37 kg milk at 3.1% protein, consuming 287 MJME, and having a metabolic BW 126 kg.

	AMINO ACID		
Amino Acid	Requirement[1]	Supply[2]	Difference
Cysteine	13.06	27.79	14.73
Valine	62.36	129.12	66.76
Phenylalanine	76.44	126.13	49.69
Arginine	58.79	132.03	73.24
Histidine	36.22	46.32	10.10
Isoleucine	88.13	120.16	32.03
Leucine	139.26	185.11	45.85
Lysine	127.63	165.41	37.78
Methionine	40.56	53.35	12.79
Threonine	71.77	117.36	45.59

[1] Estimated as the sum of the maintenance and milk production requirements.

[2] Estimated as microbial amino acid supply multiplied by its availability (0.85) plus undegraded feed amino acid supply multiplied by its availability (0.7).

Statistical Analysis

Data were analyzed using least square mean and general linear models of SAS (62). With the following model

$Y = \mu + T + P + T*P + C(T*P) + Tm + T*Tm + T*P*Tm + \epsilon$

where Y is the dependent variable, μ the mean, T the treatment, P the parity, T*P the treatment by parity interaction, C(T*P) cow nested within treatment and parity (error term 1), Tm time for repeated measures, T*Tm treatment by time interaction, T*P*Tm treatment by parity by time interaction, and ϵ the residual error (error term 2). Significance was declared at $P < .05$. Due to the relatively large number of comparisons in the analysis over time, Bonferroni's rule was adopted to maintain the observational α at .05 (69). Initially analysis was by time, with primiparous and multiparous animals separate. If primiparous and multiparous animals behaved in the same manner, the data were pooled for analysis. Similarly, if there was no time by treatment interaction, data were pooled over time for analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1

Insacco DM Disappearance

The insacco study showed a 45% degradation of methionine over 12 h, but no significant protection of lysine and threonine (Figure 1). Upon investigation it was discovered that a different fat had been used to encapsulate the lysine and threonine products. The resulting difference in final product was very marked and serves to illustrate the importance of product control.

Rumen Parameters

The pH (Figure 2), NH3-N (Figure 3), acetate (Figure 4), propionate (Figure 5), butyrate (Figure 6), valerate (Figure 7), isobutyrate (Figure 8), isovalerate (Figure 9), and total volatile fatty acid (Figure 10) profiles of the rumen digesta were not significantly ($P > .05$) affected by feeding the RPAA. Rumen microbial populations were not significantly ($P > .05$) affected by RPAA (Table 8). Ruminal DM rate of passage and liquid dilution rate were not affected by supplemental RPAA.

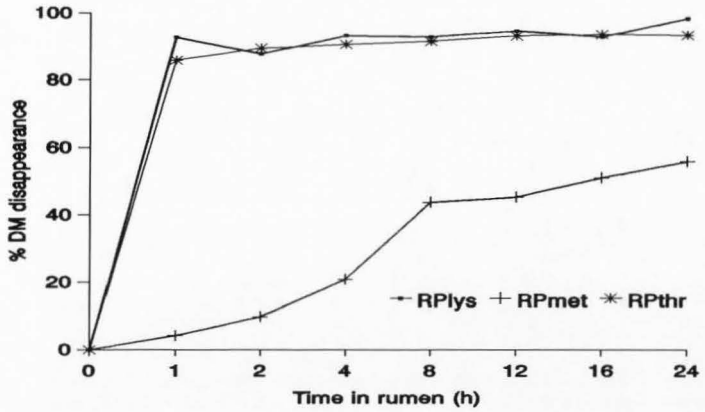

Figure 1. Insacco DM disappearance of rumen-protected lysine (RPlys), methionine (RPmet), and threonine (RPthr).

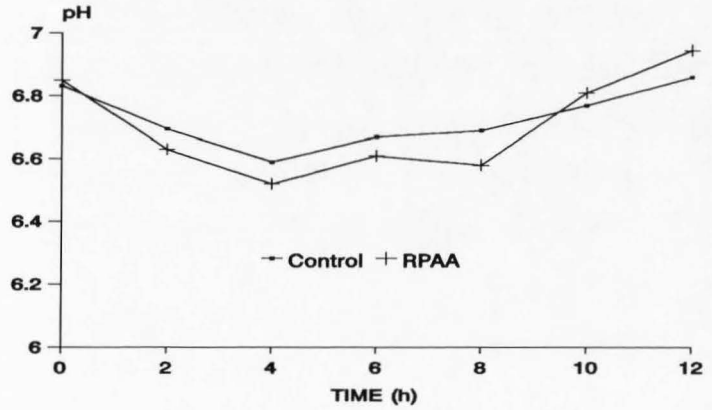

Figure 2. Effect of rumen-protected amino acids (RPAA) on rumen pH.

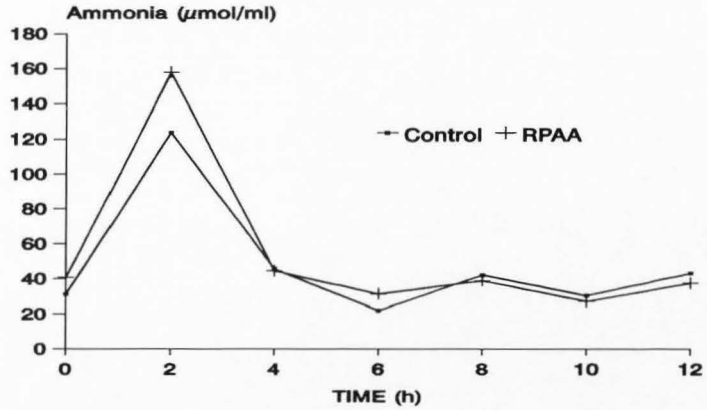

Figure 3. Effect of rumen-protected amino acids (RPAA) on rumen ammonia.

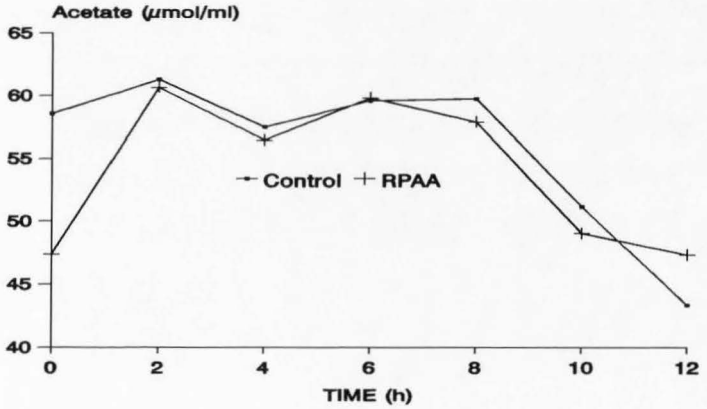

Figure 4. Effect of rumen-protected amino acids (RPAA) on rumen acetate.

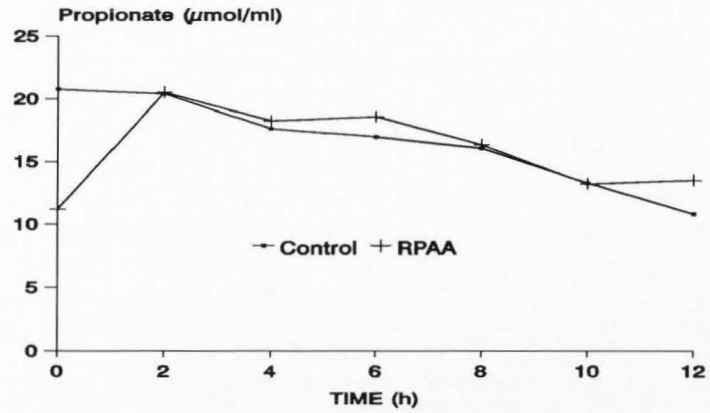

Figure 5. Effect of rumen-protected amino acids (RPAA) on rumen propionate.

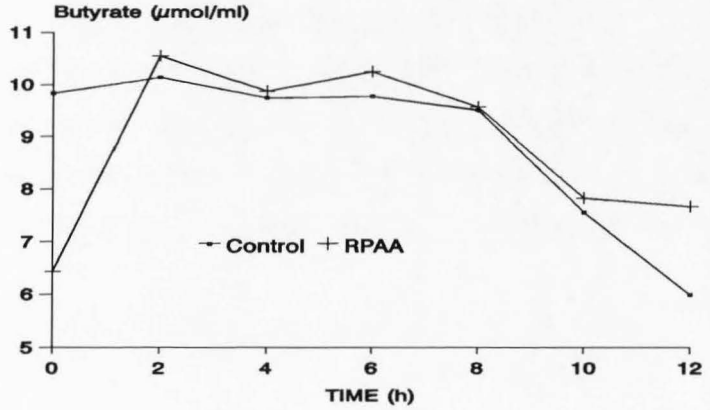

Figure 6. Effect of rumen-protected amino acids (RPAA) on rumen butyrate.

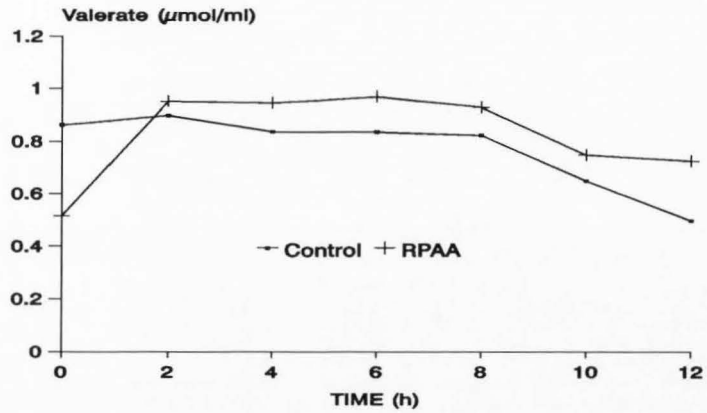

Figure 7. Effect of rumen-protected amino acids (RPAA) on rumen valerate.

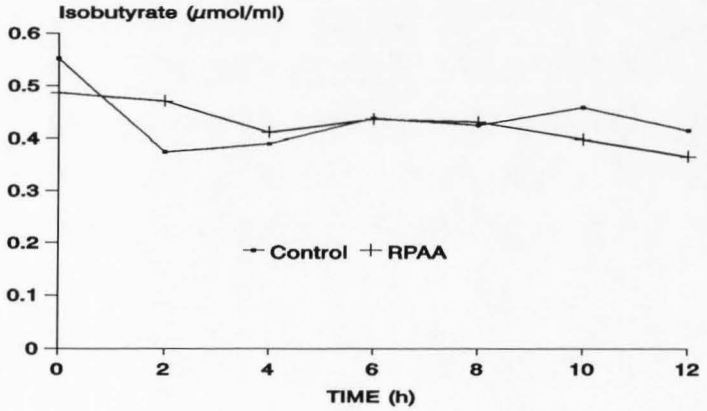

Figure 8. Effect of rumen-protected amino acids (RPAA) on rumen isobutyrate.

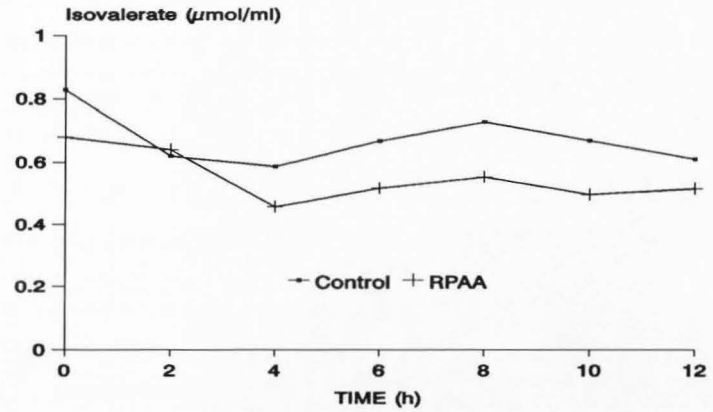

Figure 9. Effect of rumen-protected amino acids (RPAA) on rumen isovalerate.

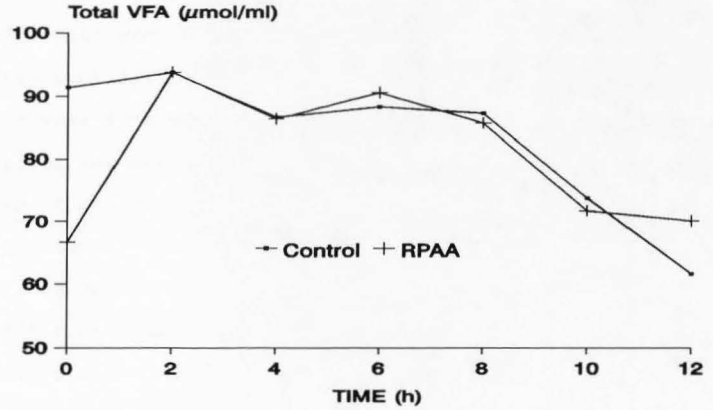

Figure 10. Effect of rumen-protected amino acids (RPAA) on rumen total volatile fatty acids.

TABLE 8. Effect of rumen-protected amino acids (RPAA) on rumen microbial populations (Log_{10}CFU/ml).

Microorganism (log/ml)	Cont.	Treat.	SEM	*P*-Value
Total bacteria	14.8	9.3	5.1	0.5
Cellulolytic bacteria	9.4	8.2	2.0	0.7
Total protozoa	4.5	4.1	1.4	0.9

Duodenal Parameters

The concentrations of NH3-N and CP were not affected ($P > .05$) by treatment (Figures 11 and 12, respectively). There was a nonsignificant increase in the concentration of each of the amino acids in the duodenal digest of cows given RPAA (Table 9). This may be due to increased microbial protein synthesis associated with release of the treatment AA into the "protein starved" rumen. An increase in microbial protein in the duodenum may have masked any methionine derived from the RPMet. We would expect an increase in duodenal methionine of 10 g (product/d) * 0.35 (methionine content of product) * 0.45 (rumen DMD) = 1.58 g/d methionine. An attempt was made to partition duodenal CP into NPN, indigestible feed-N, endogenous-N, microbial-N (using RNA as a microbial marker), and RPAA. However, the high variation, masked any significant differences.

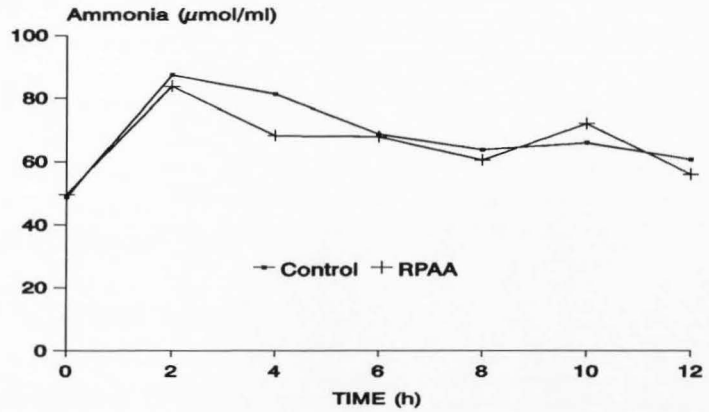

Figure 11. Effect of rumen-protected amino acids (RPAA) on duodenal ammonia.

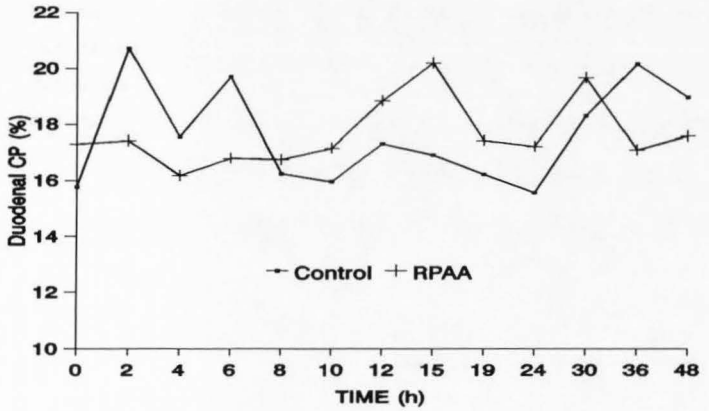

Figure 12. Effect of rumen-protected amino acids (RPAA) on duodenal CP.

TABLE 9. Effect of rumen-protected amino acids (RPAA) on duodenal amino acid concentrations (μmol/g DM).

Amino acid	Control	Treatment	SEM	*P*-Value
Alanine	101.7	116.1	6.3	0.16
Arginine	28.0	31.5	2.3	0.34
Asparagine	103.9	119.2	6.7	0.17
Glutamine	117.3	132.9	10.3	0.33
Glycine	161.8	211.9	17.0	0.09
Histidine	15.7	18.0	1.1	0.22
Isoleucine	46.8	54.2	3.3	0.17
Leucine	71.3	81.9	5.0	0.20
Lysine	66.6	75.8	4.5	0.21
Methionine	13.6	27.0	5.8	0.17
Phenylalanine	32.3	37.1	2.6	0.25
Proline	44.6	51.4	5.0	0.38
Serine	58.1	66.5	3.7	0.17
Threonine	55.6	64.2	3.6	0.15
Tyrosine	22.7	26.4	2.2	0.29
Valine	59.9	69.8	4.2	0.16

Experiment 2

The nutrient composition of the TMR is shown in Table 2. Crude protein 17.1%, ADF 26.8%, and NDF 51.4% were 1.1, 5.8, and 23.4 percentage units above minimum NRC recommendations, respectively (51).

The overall mean BW at 475 kg was approximately 25% less than the 630 kg pretrial estimate. This was partly due to the inclusion of first parity animals in the experimental estimate but not in the pretrial estimate. In addition, multiparous animals were in relatively poor condition at parturition.

Overall mean DMI (Table 10) of 17 kg/d was approximately 23% less than the 23 kg/d pretrial estimate. This was largely due to the exclusion of first parity animals, and early lactation (< 4 wk) data from the pretrial estimate, but not the experimental estimate. The differences in DMI and BW are of the same order of magnitude and resulted in a DMI of 3.75% BW.

Production Data

DMI (Figure 13) of first parity animals did not differ ($P > .05$) with treatment. Supplemental RPAA decreased DMI in multiparous animals (18.4 vs 20.7 kg/d, for treatment and control, respectively), with the difference being significant ($P < .05$) for d 16-25, and

TABLE 10. Effect of rumen-protected amino acids (RPAA) on production parameters in lactating Holstein cows.

Item	Control	Treatment	SEM	P-Value
DMI, kg/d	18.2	16.6	0.6	0.059
Milk, kg/d	33.4	33.0	1.48	0.900
Milk fat, %	3.52	3.55	0.12	0.838
Milk fat, kg/d	1.18	1.08	0.06	0.262
Milk protein, %	2.99	3.06	0.04	0.219
Milk protein, kg/d	1.01	0.94	0.05	0.303
Milk lactose, %	4.91	4.95	0.04	0.511
Milk lactose, kg/d	1.68	1.53	0.08	0.191
Milk SNF, %	8.79	8.88	0.09	0.474
Milk SNF, kg/d	3.00	2.75	0.13	0.183
Body weight, kg	479	471	9.9	0.587
DIGESTIBILITY[1]				
ADF, %	57.5	55.9	2.4	0.639
NDF, %	65.9	65.7	1.8	0.933
CP, %	74.2	73.0	1.4	0.561
DM, %	71.8	71.3	1.4	0.812

[1] Total tract apparent digestibility, wk 10 only.

again for d 31-35. This result was contrary to expectations and suggests a detrimental excess of one or more of the supplemental amino acids. Satter et al. (64) found a significant decrease in DMI when infusing DL-methionine at > .6% DMI, but no significant effect at lower levels. However, the experimental conditions used were highly variable and consequently lacked any power to detect other than gross effects. They did find numerical reductions in DMI at the lowest level of DL-methionine infused (56 g/d). In our experiment, we offered a supplement of 92 g RPmet and 43 g RPlys products (each product containing a minimum 50% of the appropriate AA, manufacturers guaranteed analysis). Assuming 100% consumption of the supplement, 100% rumen-protection of the RPAA, and 100% release of the RPAA in the abomasum, the animals received 46 g DL-methionine and 21.5 g lysine. In fact consumption of supplemental RPAA was rarely 100% and we assumed a 50% ruminal loss of AA (as suggested for methionine in the insacco study in experiment 1), resulting in an estimated 23 g of supplemental DL-methionine/d and 10.8 g of lysine. The actual levels of DL-methionine supplied to the small intestine are thought to be below those suggested to have detrimental effects on DMI. Rogers et al. (60) supplemented DL-methionine in amounts up to 28 g/d; the

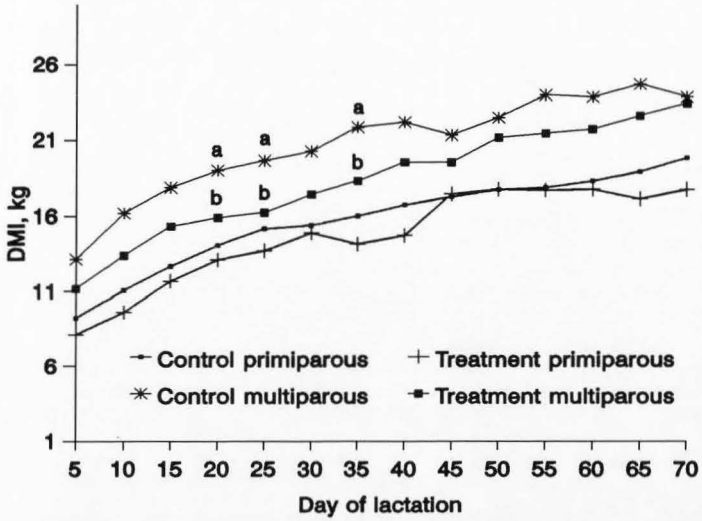

Figure 13. DMI (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ ($P < .05$).

results showed no effect on DMI. The results of Papas et al. (57) showed an increase in DMI when supplementing up to 29.4 g/d RPmet to dairy cows in early lactation.

There are two ways by which actual amount of supplemental AA reaching the small intestine could have been significantly higher than expected: Firstly, the RPAA products used in trial 1 and trial 2 were manufactured at different times. That significant

differences in the products existed is indicated by the AA concentration in the product, 35% and >49% for studies 1 and 2, respectively (manufactures analysis). Further, the product used in experiment 2 had a minimum of 50% AA, with the actual content being unknown. Secondly, the 50% loss of AA in the rumen, predicted by trial 1, may significantly overestimate the loss of AA under conditions in the lactating animal. Studies evaluating AA toxicity have generally concentrated on methionine because it is thought to be one of the more toxic (63). It is possible that either the lysine itself or the lysine together with the methionine are interacting to produce the reduction in DMI in cattle. However, numerous studies have fed similar quantities of lysine, alone or with methionine, without detriment to DMI (10, 11, 60, 61). None of the studies mentioned evaluated total AA concentration in the intestinal tract. This is presumably the important factor in any toxic effect. Though similar levels of AA have been supplemented in previous studies, it seems probable that the combination of feed, microbial, and RPAA provided in this experiment were sufficient to produce the detrimental effects seen in DMI. Since there was no significant similar effect on DMI among primiparous animals, it may be due to the combination of lower DMI in mature cows and their different AA requirements associated

with continuing tissue growth.

Milk production (Figure 14) was not different due to treatment among primiparous animals. Supplemental AA reduced milk production by approximately 6 kg/d among multiparous animals (34.7 vs 40.8 kg, for treatment and control animals, respectively). The reduction in milk production was apparent within 2 wk of commencing the trial and was fairly constant throughout the remainder of the experimental period. The milk production pattern is similar to that seen with DMI. The 2.3 kg/d reduction in DMI would have provided 3.85 Mcal/d, sufficient to produce around half the 6 kg/d difference due to treatment. The remaining difference in milk production is probably due to nonsignificant differences in BW (Figure 15).

The more erratic nature of weekly BW measurements masks any statistical differences in BW. However, there are numerical differences in BW (Figure 15) which are consistent with the DMI and milk production data, suggesting that RPAA-supplemented, multiparous animals lost more weight than control animals.

Supplemental AA did not significantly affect the percent milk fat, SNF, lactose, protein, casein or whey (Table 10). However, supplemental AA resulted in a significant reduction in % milk NPN among multiparous animals (Figure 16). This is thought to be an indirect

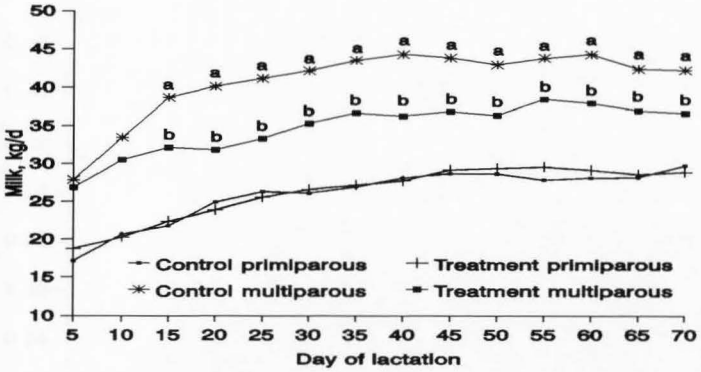

Figure 14. Milk production for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ ($P < .05$).

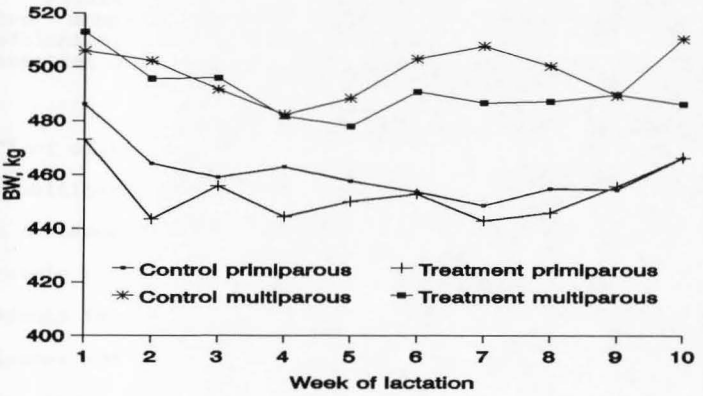

Figure 15. BW (kg) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine.

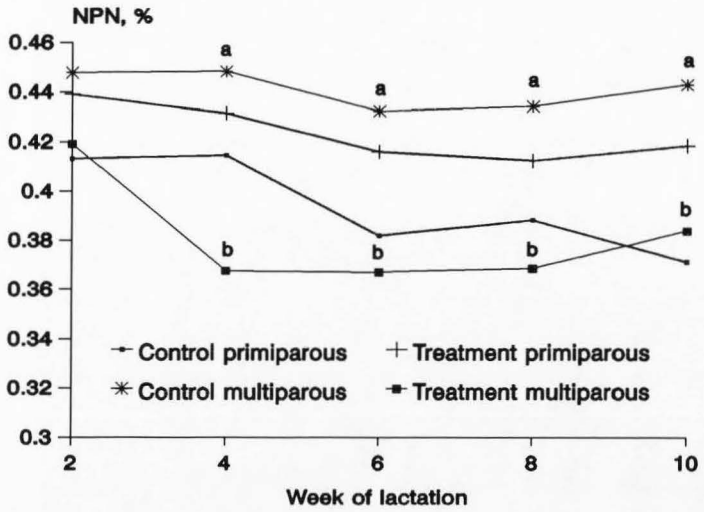

Figure 16. Milk NPN% for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ (P <.05).

effect of the supplemental AA on DMI. Thus, reduced DMI by multiparous animals receiving supplemental AA resulted in a reduced protein intake. This in turn results in less protein breakdown in the rumen and liver, leading to reduced ammonia levels in the rumen, and urea in the tissues and milk.

Among multiparous animals, treatment effects on milk production, without differences in concentration of milk

components (except NPN), resulted in there being differences in yield of milk components: Yield of lactose (Figure 17), SNF (Figure 18), and protein (Figure 19) were lower for multiparous animals receiving supplemental RPAA. Milk fat yield (Figure 20) had nonsignificant ($P > .05$) differences with similar patterns in the other milk components. The reason milk fat yield was not statistically different is thought to be due to the ameliorating effect of increased body fat depletion among treatment animals.

Yield of 4% FCM (Figure 21) among multiparous animals was significantly decreased at 8 wk with RPAA supplementation. The difference in FCM was less marked than in milk itself, presumably due to the ameliorating effect of the nonsignificant increase in BW loss among treatment animals, resulting in compensatory milk fat production. There was no effect of RPAA supplementation on total tract apparent digestibility of ADF, NDF, CP, and DM during wk 10 of the trial (Table 10).

Primiparous animals did not show any significant ($P < .05$) difference due to supplemental AA. Primiparous animals showed a similar trend to multiparous animals for DMI, with supplemented animals eating less. There was no trend apparent among primiparous animals for milk, milk component yield, or percent milk components.

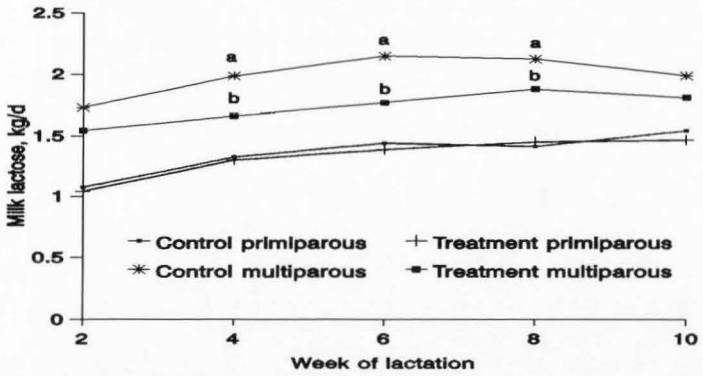

Figure 17. Milk lactose production (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ (P <.05).

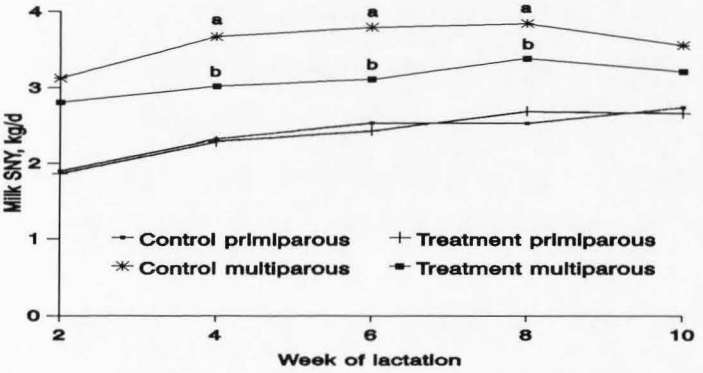

Figure 18. Milk SNF production (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ (P <.05).

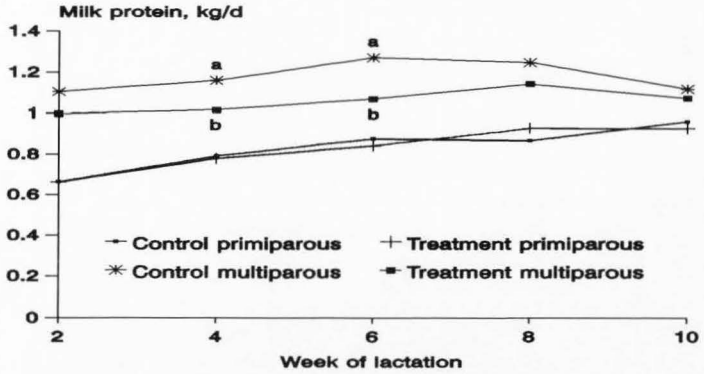

Figure 19. Milk protein production (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ (P <.05).

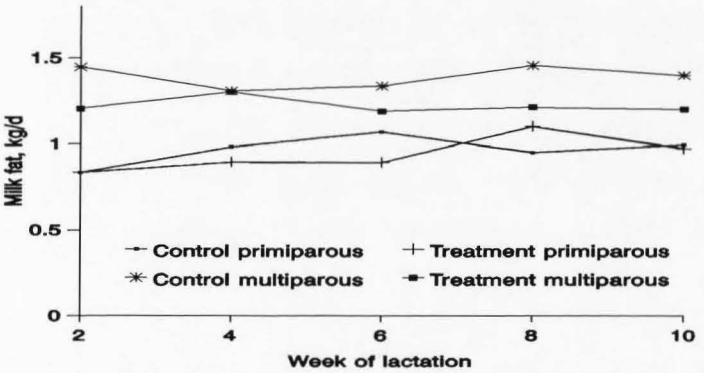

Figure 20. Milk fat production (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ (P <.05).

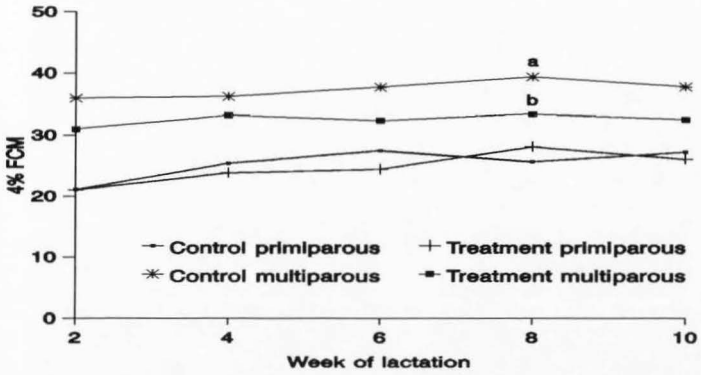

Figure 21. Four percent fat corrected milk production (kg/d) for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine. a,b = Cows of the same parity, at that time of lactation differ ($P < .05$).

CHAPTER V

CONCLUSIONS

Experiment 1

The results of the fermentation study clearly demonstrate that use of inappropriate fatty acids in the manufacture of a polymer capsule can result in failure of rumen-protection. When correct manufacturing procedures were followed (RPmet only), 40 to 50% of the protected product was degraded in the rumen under the conditions of this trial. As expected, there were no significant ($P < .05$) effects of feeding RPAA on rumen function.

Despite the use of a semipurified ration we were unable to detect any significant increase in duodenal methionine. This was because of the low effective dose (1.75 g/d methionine to the duodenum) and the high variation of these types of measurements.

Experiment 2

The RPAA products used in experiment 2 were from a different batch than those used in experiment 1. The products used in experiment 2 contained a minimium of 50% AA, compared to 35% for experiment 1. Under the conditions of experiment 2, supplemental rumen-protected methionine and lysine were detrimental to multiparous

animals. The primary reason for reduced milk production is thought to be decreased DMI and thereby nutrient supply.

The levels of supplemental RPAA used in this experiment have been used by others without reducing DMI. It may be that supplemental RPAA together with the basal ration combined to produce a toxic excess of one or more AA in the small intestine, and that the excess resulted in a homeostatic reduction, possibly via hormonal intermediates, in DMI. Methionine toxicity studies in chickens (31) showed excess methionine decreases growth and hematocrit values, and increases requirements for glycine and copper.

Many of the effects seen in this trial were apparent within the first 2 wk; this supports the idea that labile protein reserves are limited. This should minimize carry-over effects between periods and suggests crossover designs may be more efficient at testing for AA effects.

Primiparous animals appear to have significantly different responses to RPAA, presumably associated with differences in requirements, production, and DMI.

Future research should concentrate on alleviating specific AA deficiencies. In order to do this, ration protein concentration should be below NRC recommendations. Using the procedures similar to those outlined in this

paper, AA requirements and availabilities can be estimated. Alternatively, computer modeling of rumen function may provide the necessary data. Since the commencement of this project there has been at least one software program released for this purpose (24). Primiparous and multiparous animals have different requirements and should be examined separately. A crossover design using experimental periods of 4 to 6 wk should provide a more sensitive test and may allow fewer animals to be used.

Not enough is known for widespread commercial use of RPAA. In the near future its use is likely to be restricted to high-producing cows in well managed herds. However, if producers or nutritionists want to try these products, they should be aware of the following: If protein is not limiting, there will be no benefit from supplemental AA. A small amount (5 to 10 g/d) of an AA that was deficient may provide a significant improvement in milk protein yield. An excess or an imbalance of AA can reduce performance. The results of this trial indicate that effects, both beneficial and detrimental, of supplemental RPAA should be noticeable within 2 wk of application.

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APPENDICES

TABLE 11. Least square mean DMI kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 13).

	Multiparous			Primiparous		
DAY	Cont.	RPAA	SEM	Cont.	RPAA	SEM
1	13.1	11.2	.70	9.2	8.1	.90
6	16.2	13.4	.70	11.1	9.6	.90
11	17.9	15.3	.70	12.7	11.7	.90
16	19.0	15.9	.70	14.1	13.1	.90
21	19.7	16.2	.70	15.2	13.7	.90
26	20.3	17.4	.70	15.4	14.9	.90
31	21.9	18.3	.70	16.0	15.2	.90
36	22.2	19.6	.70	16.8	15.7	.90
41	21.3	19.5	.70	17.6	17.5	.90
46	22.5	21.1	.70	17.8	17.8	.90
51	24.0	21.4	.70	17.9	17.7	.90
56	23.8	21.7	.70	18.3	17.8	.90
61	24.7	22.6	.88	18.8	17.1	1.00
66	23.9	23.4	.88	19.8	17.8	1.04

TABLE 12. Least square mean milk kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 14).

	Multiparous			Primiparous		
DAY	Cont.	RPAA	SEM	Cont.	RPAA	SEM
1	27.9	26.9	0.9	17.1	18.8	1.2
6	33.5	30.5	0.9	20.7	20.3	1.2
11	38.7	32.2	0.9	21.8	22.4	1.2
16	40.1	31.8	0.9	24.9	23.9	1.2
21	41.2	33.3	0.9	26.3	25.6	1.2
26	42.2	35.3	0.9	26.1	26.2	1.2
31	43.5	36.7	0.9	27.0	27.2	1.2
36	44.2	36.3	0.9	28.0	29.7	1.2
41	43.8	36.9	0.9	28.7	29.2	1.2
46	43.0	36.4	0.9	28.7	29.4	1.2
51	43.8	38.4	0.9	27.9	29.6	1.2
56	44.3	38.0	0.9	28.1	29.2	1.2
61	42.4	37.0	1.1	28.2	28.6	1.3
66	42.3	36.6	1.3	29.8	28.9	1.4

TABLE 13. Least square mean BW kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 15).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
1	505.9	512.9	15.8	486.3	473.1	10.1
2	502.3	495.7	15.8	464.4	443.6	10.1
3	491.7	495.9	15.8	459.1	455.9	10.1
4	482.3	481.6	15.8	463.0	444.4	10.1
5	488.4	477.9	15.8	457.7	450.1	10.1
6	502.9	490.8	15.8	453.6	452.9	10.1
7	507.7	486.6	15.8	448.6	442.9	10.1
8	500.3	487.1	15.8	454.7	445.9	10.1
9	489.3	489.6	15.8	454.5	455.6	10.1
10	510.3	486.2	15.8	466.4	466.4	10.1

TABLE 14. Least square mean NPN percent in milk from primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 16).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	.448	.419	.011	.413	.439	.013
4	.448	.368	.011	.405	.432	.013
6	.432	.367	.011	.382	.416	.013
8	.434	.369	.011	.388	.413	.013
10	.443	.384	.013	.371	.419	.018

TABLE 15. Least square mean milk lactose yield kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 17).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	1.73	1.55	.056	1.08	1.04	.069
4	1.99	1.66	.052	1.33	1.30	.069
6	2.15	1.77	.052	1.44	1.39	.066
8	2.12	1.88	.052	1.41	1.45	.066
10	1.98	1.81	.086	1.56	1.47	.100

TABLE 16. Least square mean SNF yield kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 18).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	3.13	2.81	.104	1.90	1.86	.126
4	3.67	3.02	.096	2.32	2.29	.126
6	3.79	3.11	.096	2.53	2.43	.127
8	3.84	3.39	.096	2.53	2.68	.127
10	3.56	3.21	.140	2.74	2.66	.185

TABLE 17. Least square mean milk protein yield kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 19).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	1.11	1.00	.032	.66	.66	.040
4	1.16	1.02	.030	.79	.78	.040
6	1.27	1.07	.030	.88	.84	.038
8	1.25	1.14	.030	.87	.93	.038
10	1.18	1.07	.045	.96	.93	.058

TABLE 18. Least square mean milk fat yield kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 20).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	1.45	1.21	.087	.84	.84	.106
4	1.31	1.30	.083	.98	.90	.106
6	1.34	1.19	.083	1.07	.90	.103
8	1.46	1.21	.083	.95	1.10	.103
10	1.40	1.20	.146	1.00	.98	.155

TABLE 19. Least square mean 4% fat corrected milk yield kg/d for primiparous and multiparous cows supplemented with rumen-protected lysine and methionine (Figure 21).

	Multiparous			Primiparous		
Week	Cont.	RPAA	SEM	Cont.	RPAA	SEM
2	36.0	31.0	1.7	21.1	21.0	1.8
4	36.2	33.2	1.5	25.4	23.9	1.8
6	37.7	32.4	1.5	27.5	24.4	1.8
8	39.4	33.4	1.5	25.7	28.1	1.8
10	37.8	32.6	2.4	27.2	26.1	2.7

CURRICULUM VITAE

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EDUCATION

Ph.D. Animal Nutrition. Expected completion date spring 1993. Utah State University. Dissertation title: Amino acid nutrition of the dairy cow.

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WORK EXPERIENCE

January 1990 to present. Graduate research assistant, Dept, of Animal, Dairy and Veterinary Science, Utah State University, Logan Ut. Responsibilities include: Design, collection and analysis of data from studies with cows, and forage. Served as teaching assistant for courses in mineral metabolism and animal nutrition.

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April to September 1983. Research assistant, National Institute for Research into Dairying, Shinfield Road, Reading, Berkshire, England. Responsibilities: Assist in research into the allergic response of the calf to milk replacers containing soybean protein.

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PUBLICATIONS

Batallas, C. E., R. L. Bowman, D. P. Dawson, and M. J. Arambel. 1991. Effects of feeding a high level of rumen protected fat with bypass protein with or without niacin on milk production response in the early to mid lactation Holstein. J. Dairy Sci. 74(supple. 1):253(Abstract).

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